#11
Harry
Feb. 23, 02

IN THE UNITED STATES PATENT OFFICE

APPLICATION OF)
)
LARS E. SUNDSTROM)
)
SERIAL NUMBER 09/581,397)
)
FILED: October 2, 2001)
)
TITLE: NEUROPROTECIVE AGENTS)

DECLARATION UNDER RULE 1.132

I, Lars E. Sundstrom, hereby declare the following:

I received a Bachelor of Science Degree in Biochemistry from Oxford University in 1981; etc: & a DPhil in Physiological Psychology from Oxford Uni. in 1988

I have been employed by the University of Southampton since 1995;

From 1988 through 2001 I have engaged in research relating to neuronal dysfunction and neurodegeneration etc. A copy of my curriculum vitae is attached hereto.

I have conducted and/or supervised a series of tests to compare the purity of arginine-spermidine and lysine-spermidine prepared in accordance with the procedure described in WO 93/12777 and in accordance with the synthesis procedure described in the above-referenced application (09/581,397).

Under my direction, the methods described in WO 93/12777 were reproduced exactly in an attempt to synthesize arginine-spermidine and lysine-spermidine, which are analogous to compounds A and B in the above-referenced application.

The products for the methods defined in WO 93/12777 were examined by mass spectrophotometry, and the results are attached hereto.

The mass spectrophotometry results for the products prepared as described in the WO 93/12777 show significant peaks for various starting materials, but do not show any trace of the alleged products, arginine-spermidine or lysine-spermidine.

In my opinion, the results show that arginine-spermidine and lysine-spermidine, if present at all, are present in the final product in an amount less than 1%.

Neuroprotection experimental procedures identical to those described in the above-referenced application were performed under my direction. Cultures exposed to hypoxia in the absence of arginine-spermidine and lysine-spermidine have neuronal damage detectable in 44.6 ± 3.7% of the CA1 pyramidal cell layer (mean ± sem, n = 8). Cultures exposed to either 40 microliters or 100 microliters of arginine-spermidine prepared in accordance with the methodology described in WO 93/12777 had significantly greater damage in CA1 (99.4 ± 0.6% and 98.2 ± 1.1% respectively; n = 8 & 7; $p < 0.001$ verses control for both).

A similar pattern of damage occurred with lysine-spermidine prepared in accordance with the methodology described in WO 93/12777 (99.7 ± 0.2% damage, n = 8, $p < 0.001$ verses control for 40 microliters; and 98.3 ± 0.2%, n = 8, $p < 0.001$ verses control for 100 microliters).

Further, for the control, damage was confined to neuronal cells, whereas in both the arginine-spermidine and lysine-spermidine prepared in accordance with the methodology described in WO 93/12777 both neuronal and glial cells were damaged, indicating that the products prepared in accordance with the teachings of WO 93/12777 contained compounds that were highly toxic to all cells in the cultures.

It is my opinion that the results demonstrate that the products prepared in accordance with the methodology described in WO 93/12777 are not pure, and are extremely toxic, and therefore are not encompassed within the scope of the claims of the above-referenced application.

Further, given the fact that the compounds produced in accordance with the teachings of WO 93/12777 are extremely toxic, as demonstrated by the facts stated above, it would not be obvious to attempt to prepare the claimed substantially pure compounds of the above-referenced application using the methodology described in WO 93/12777.

I have read U.S. Patent Nos. 5,242,947 and 5,432,202, and WO 91/00853 and have determined that the methods for preparing arginine-spermidine and lysine-spermidine described in these documents are substantially the same as those described in WO 93/12777.

Accordingly, it is also my opinion that U.S. Patent Nos. 5,242,947 and 5,432,202, and WO 91/00853 disclose processes for synthesizing compounds which are neither pure nor non-toxic, and which would not anticipate or suggest the substantially pure compounds claimed in the above-referenced application.

All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further, these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

24.10.2001
Date

Lars E. Sundström

University of Southampton

Div. of Clinical Neurosciences
University of Southampton
Biomedical Sciences Bldg.
Bassett Crescent East
Southampton SO16 6YD.
United Kingdom.

Phone (+44) (0)380 59/806
Fax (+44) 02380 597809
E-mail les@soton.ac.uk

Dr. Lars Eric Sundstrom

Personal Information

Marital status: Married , 2 children.
Nationality : American and Swedish.

Present Appointment

Senior Lecturer in Neurosciences
University of Southampton, UK.

National grading : ACLS 22
Date of appointment : 1.10.95
Faculty : Medicine Health and Biological Sciences

Previous Appointments

Dates	Appointment
1993-1995	Associate Professor, CNRS. EP74 Unite de Biologie Moleculaire des Interactions Neuronales, University of Bordeaux II, France
1990-1993	University Lecturer In Pharmacology, Dept. of Physiology and Pharmacology, University of Southampton, England.
1988-1990	Sir Desmond Pond Research Fellow, Epilepsy Research Foundation, Dept. of Experimental Psychology, Oxford University
1982-1983	Research Assistant/Biochemist, Hans Wilsdorf Laboratory of Immunology, University Of Geneva, Switzerland.

Education

Date	Title of Award	Subject	Class	Awarding Body
1988	M.A./D.Phil.	Physiological Psychology	----	Magdalen College, Oxford University.
1981	B.A.	Biochemistry	2 (-)	Magdalen College Oxford University.
1977	International Baccalaureate		----	International School of Geneva.

University of Southampton

Publicati ns

Full peer reviewed publications:

Sundstrom L.E., Brana C., Gatherer M., Mepham J., Rougier A., Somatostatin and neuropeptide-Y mRNA synthesiszing neurons in the fascia dentata of humans with temporal lobe epilepsy in relation to hilar neurons loss Brain (2001) 124 688-697.

Brana C., Benham C.D., Sundstrom L.E., Neuroprotective role of monocarboxylate transport during glucose deprivation in slice cultures of rat hippocampus J. Physiol. (2001) 531 459-466.

Wilde G., Pringle A.K., Sundstrom L.E., Mann D., Iannotti F., Attenuation and augmentation of ischaemia-related neuronal death by tumour necrosis factor alpha in-vitro. Eur. J. Neurosci. (2000) 12(11), 2000, 3863-70.

Brana C., Biggs T.E., Mann D.A., Sundstrom L.E., A Macrophage hippocampal slice co-culture system: Application to the study of HIV induced brain damage.(1999) J. Neurosci Methods 90: 7-11.

Brana C., Biggs T.E., Barton C.H., Sundstrom L.E., Mann D.A., A soluble factor produced by macrophages mediates the neurotoxic effects of HIV-1 tat in vitro. AIDS (1999) 13:1443-1452

Brana C., Benham C.D., Sundstrom L.E., Calpain activation and inhibition in organotypic hippocampal slice cultures deprived of oxygen and glucose. Eur. J. Neurosci. (1999) 11:2375-2384.

El Bahh B., Lurton D., Coussemacq M., Sundstrom L., Rougier A., Seizure-linked hippocampal plasticity and protection against excitotoxicity: possible role of neuropeptide-y. Neurochirurgie (1998) 44 31-7

Gatherer M., Sundstrom L.E., Mossy fibre innervation is not required for the development of kanic acid toxicity in organotypic hippocampal slice cultures. Neurosci. Lett (1998) 253: 119-122

Gray W.P., Sundstrom L.E., A unilateral intracerbroventricular kainic acid injection bilaterally increases neurogenesis in the dentate gyrus of the adult rat. Brain Res. (1998) 790: 52-59

Lurton D., Sundstrom L.E., Rougier A., Granule cell dispersion is correlated with early epileptic events in human temporal lobe epilepsy. J. Neurol. Res. (1998) 154: 133-136

Sundstrom L.E., Sundstrom K.E., and Mellanby J.H., A new protocol for the transmission of physiological signals by digital telemetry. J. Neurosci. Methods (1997) 77:55-60.

University of Southampton

Sundstrom L.E., Epileptic brain damage a consequence and a cause of seizures- Editorial Comment. Neuropath. App. Neurobiol (1997) 23(3) 201-202.

Lurton D., Sundstrom L.E. Branna C., Vital A., Bloch B., Rougier A., Possible mechanisms inducing granule cell dispersion in humans with temporal lobe epilepsy. Epilepsy Res. (1997) 26(2):351-361.

El Bahh B., Lurton D., Sundstrom L.E., Rougier A., Induction of tolerance and mossy fibre neuropeptide-Y expression in the contralateral hippocampus following a unilateral intrahippocampal kainic acid injection in the rat. Neurosci. Lett. 227 (1997) 135-139.

Pringle A.K., Angewalla R., Wilde G.J.C., Mepham J., Sundstrom L.E., Iannotti F., Induction of 72kDA heat-shock protein following sublethal oxygen deprivation in organotypic hippocamapl slice cultures. *Neuropath Appl. Neurobiol* (1997) 23: 289-298.

Pringle A.K., Iannotti F., Wilde G.J.C., Chad J.E., Seeley P.J., Sundstrom L.E. Neuroprotection by both NMDA and non-NMDA receptor antagonists in in-vitro ischaemia. Brain Res. (1997) 755: 36-46.

Gonon F., and Sundstrom L.E., Excitatory effects of dopamine released by impulse flow in the rat nucleus accumbens in-vivo. Neuroscience (1996) 75:1 13-18.

Best N., Sundstrom L.E., Mitchell J. and Wheal H., Pre-exposure to subtoxic levels prevents kainic acid lesions in organotypic hippocampal slice cultures: Effects of kainic acid on parvalbumin-immunoreactive neurons and expression of heat shock protein 72 following the induction of tolerance. Eur J. Neurosci. (1996) 8 1209-1219.

Mitchell J. Best N., Sundstrom L.E., Wheal H., Hippocampal NPY neurons project to the fascia dentata in organotypic cultures (1996) Hippocampus (1996) 6 173-182.

Pringle A.K., Sim L., Kennedy J., Benham C.D., Ianotti F., Sundstrom L.E., Selective n-type calcium channel antagonist omega conotoxin MVIIA is neuroprotective against hypoxic neurodegeneration in organotypic hippocampal-slice cultures. Stroke. (1996) 27:11 2124-2130.

Lurton D., Cousemaq M., Barrow P., Sundstrom L.E., Rougier A., Widespread ectopic neuropeptide-Y expression in contralateral mossy fibres after a unilateral intrahippocampal kainic acid injection in the rat. Neurosci. Lett. (1996) 213 181-184.

Pringle A.K, Sundstrom L.E, Wilde G.J.C, Iannotti F., Brain Derived Neurotrophic Factor, but not neurotrophin-3, prevents ischaemia-induced neuronal cell death in organotypic rat hippocampal slice cultures. (1996) Neurosci. Lett. 211 203-206

Mitchell J., Gatherer M., Sundstrom L.E., Aberrant Timm's stained fibres in the dentate gyrus following tetanus toxin-induced seizures in the rat. Neuropath Apppl. Neurobiol. (1996) 22 129-135.

University of Southampton

Rougier A., Sundstrom L.E., Clavrie B. St-Hilaire J-M.,Labreque R., Lurton D., Bouvier G, Multiple subpial transections: A report on 7 cases. (1996) Epilepsy Research. (1996) 24 57-63.

Mitchell J., Gatherer M., Sundstrom L.E., Loss of hilar somatostatin neurons following tetanus toxin-induced seizures in the rat (1995) Acta Neuropath. 89; 425-430.

Wilde G., Sundstrom L.E., Iannotti F., Propidium Iodide as an early marker of neuronal damage in-vivo. (1994) Neurosci. Lett. 180; 223-226.

Sundstrom L.E., Mitchell J., Wheal H.V., Bilateral reorganisation of mossy fibres in the rat hippocampus after a unilateral intracerebroventricular injection of kainic acid. (1993) Brain Res. 609; 321-326.

Mitchell J., Sundstrom L.E., Wheal H.V., Microglial and astrocytic cell responses in the rat hippocampus after anintracerebroventricular kainic acid injection. (1993) Exp. Neurol., 121; 224-230.

Mitchell J., Best N., Sundstrom L.E., Wheal H.V., The use of sodium sulphide-fixed brain tissue for immunocytochemical staining of activated microglia and reactive astrocytes. (1993) Histochemistry 99; 91-94.

Sundstrom, L.E., and Mellanby, J.H., Tetanus toxin blocks inhibition in the dentate gyrus of the urethane-anaesthetized rat. (1990) Neuroscience. 38; 621-627.

Mellanby, J.H., and Sundstrom, L.E., (1989) Epilepsy, Kindling, Memory and Behaviour. In The Clinical Relevance Of Kindling. (eds.) M.Trimble and T.Bolwig. pp 103-112 (Wiley)

Sundstrom, L.E, and Mellanby, J H., (1988) Acute effects of tetanus toxin on rat hippocampal granule cells in vivo. In Proceedings Of The Eighth International Conference On Tetanus. (eds.) Nistico. G., Bizzini. B., Bytchenko. B., and Thiau.,. Pythagora Press Rome-Milan 149-153.

Dayer,J.-M., Sundstrom,L.E., Polla,B.S., Junod,A.F., Cultured human alvacolar macrophages from smokers with lung cancer : Resolution of factors that stimulate fibroblast proliferation and production of collagenase, or prostaglandin E2. (1985) Journal Of Leukocyte Biology 37; 641-649.

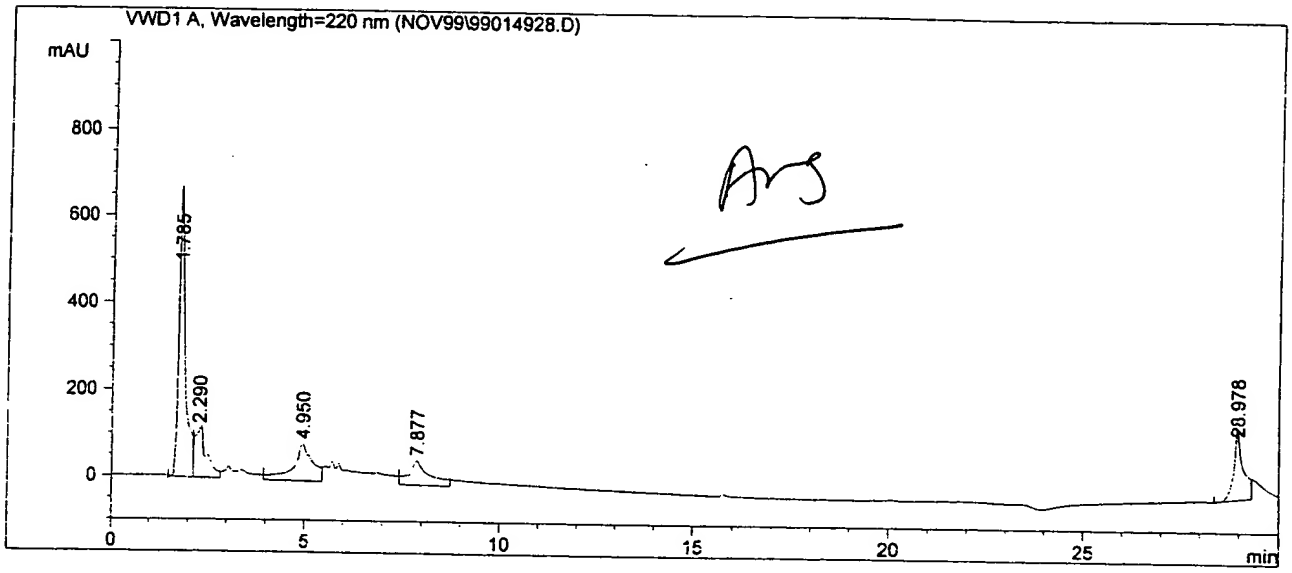
O'Neil, R.D., Filenz, M., Sundstrom, L.E., Rawlins, J.N.P. Voltammetrically monitored brain ascorbate as an index of excitatory amino acid release in the unrestrained rat. Neurosci. Lett. (1984) 52; 227-223.

Data File D:\DATA\NOV99\99014928.D Arg-Sp. Sample Name: BA-ARG

Injection Date : 22/11/99 21:55:18
Sample Name : BA-ARG
Acq. Operator : sunil rana

Seq. Line : 75
Vial : 26
Inj : 1
Inj Volume : 20 µl

Acq. Method : C:\HPCHEM\1\METHODS\LONG220.M
Last changed : 22/04/99 11:39:45 by Jen
Analysis Method : C:\HPCHEM\1\METHODS\LOIC260.M
Last changed : 23/11/99 10:11:22 by sunil rana
(modified after loading)
gradient from 0 to 100% MeCN/TFA in 12 min at 260nm (high needle position)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

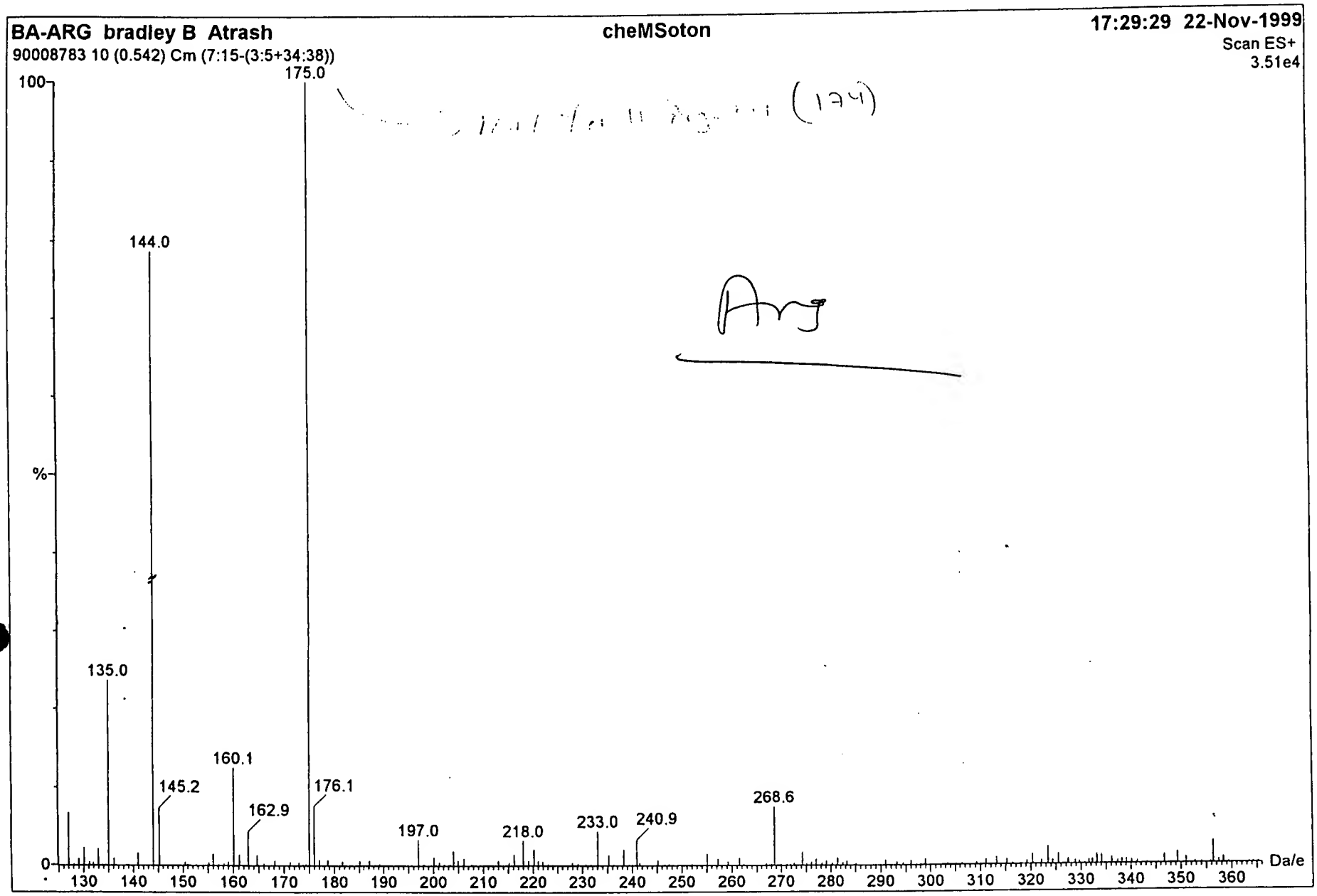
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	1.785	PV	0.2312	7639.31299	482.79102	42.6536
2	2.290	VV B	0.3215	2422.82886	112.33123	13.5277
3	4.950	VV	0.4934	3039.86841	83.73086	16.9729
4	7.877	VV	0.5299	2143.09351	54.34762	11.9658
5	28.978	VV	0.2798	2665.01489	133.30199	14.8799
Totals :				1.79101e4	866.50271	

Results obtained with enhanced integrator!

==========

*** End of Report ***

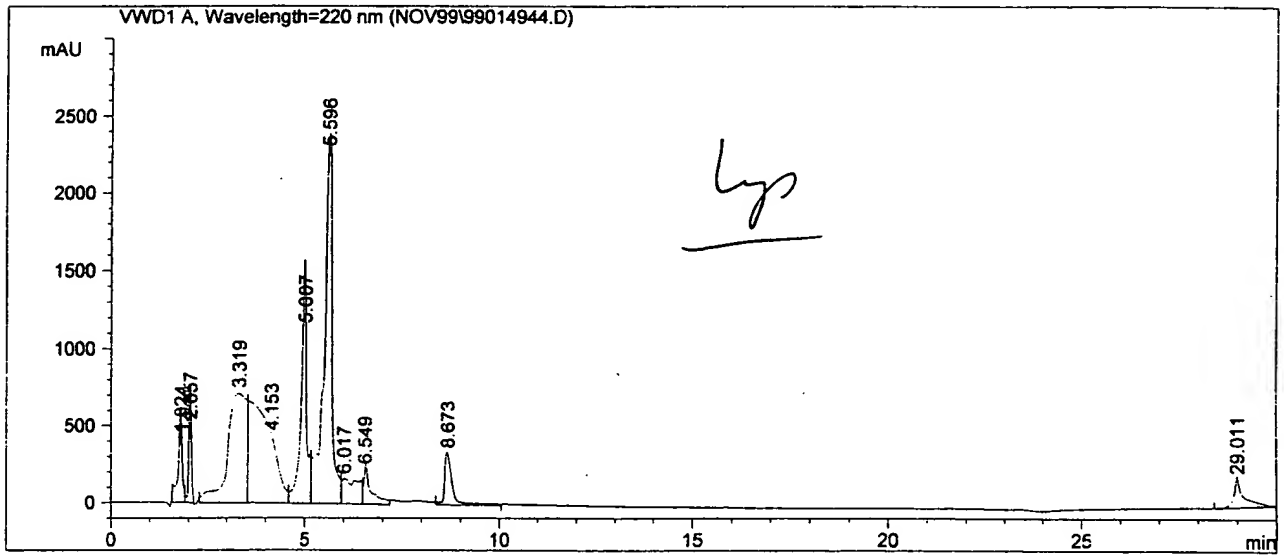

BA-ARG bradley B Atrash
90008783 10 (0.542) Cm (7:15-(3:5+34:38))
cheMSoton
17:29:29 22-Nov-1999
Scan ES+
3.51e4
100
%
0
175.0
144.0
135.0
160.1
145.2
176.1
162.9
197.0
218.0
233.0
240.9
268.6
130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320 330 340 350 360
Da/e

Data File D:\DATA\NOV99\99014944.D Sample Name: BA-LYS

Lys-Sp.

Injection Date : 23/11/99 12:05:17 Seq. Line : 9
Sample Name : BA-LYS Vial : 44
Acq. Operator : sunil rana Inj : 1
Inj Volume : 20 µl

Acq. Method : C:\HPCHEM\1\METHODS\LONG220.M
Last changed : 22/04/99 11:39:45 by Jen
Analysis Method : C:\HPCHEM\1\METHODS\LOIC260.M
Last changed : 23/11/99 14:17:21 by sunil rana
(modified after loading)
gradient from 0 to 100% MeCN/TFA in 12 min at 260nm (high needle position)



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

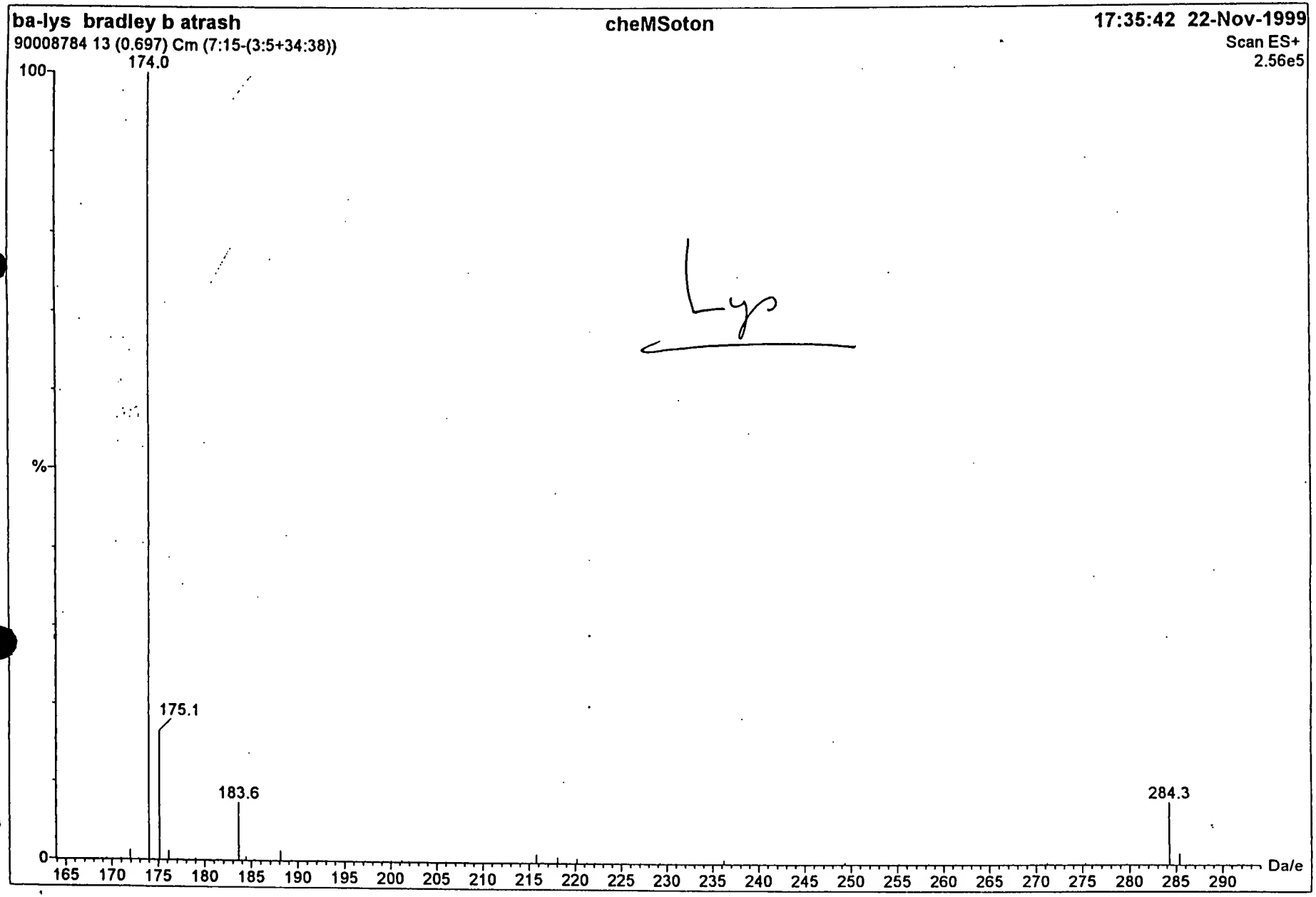
Signal 1: VWD1 A, Wavelength=220 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	1.824	BV F	0.1620	4118.27539	416.49362	3.2901
2	2.057	VV F	0.1169	3541.51245	504.96072	2.8293
3	3.319	VV B	0.5087	2.39462e4	712.30377	19.1307
4	4.153	VV B	0.9502	2.75018e4	433.26202	21.9713
5	5.007	VV F	0.2052	1.54182e4	1134.46497	12.3176
6	5.596	VV	0.2195	3.37918e4	2279.49341	26.9964
7	6.017	VV B	0.4931	4758.88916	160.86272	3.8019
8	6.549	VV	0.2522	3413.51489	225.54660	2.7271
9	8.673	VV	0.2514	5162.63281	331.85999	4.1244

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
10	29.011	VBA	0.2982	3518.87769	179.79948	2.8112
Totals :				1.25172e5	6379.04730	

Results obtained with enhanced integrator!

*** End of Report ***


ba-lys bradley b atrash
90008784 13 (0.697) Cm (7:15-(3:5+34:38))
cheMSoton
17:35:42 22-Nov-1999
Scan ES+
2.56e5
100
%
0
174.0
175.1
183.6
284.3
Lys
165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290
Da/e

	Cell area	CA1 ar a	CA3 ar a	DG area	Total cell d	CA1 damag	CA3 damag
Hyp xia Contr l							
2a	126720	40316	36779	49625	105367	18782	21629
2b	135399	38997	39045	57357	74580	20818	11741
2c	118165	36329	34617	47219	92240	18518	31320
2d	126512	36666	37716	52130	62640	14778	9913
3a	116350	27405	37340	51605	61886	10287	6210
3b	138376	44166	40760	53450	88388	26232	10445
3c	125728	40956	41623	43149	70983	16271	12567
3d	139079	43063	42384	53632	67952	12378	8030
40µl NY Arg-Sp							
5a	126538	34627	35121	56790	123996	34619	32762
5b	137974	30989	44729	62256	136122	30964	44719
5c	126777	33021	33928	59828	126770	33021	33928
5d	142006	37517	41853	62636	140437	35948	41853
6a	124550	33596	40104	50850	124523	33569	40104
6b	123924	36827	39145	47952	123510	36827	39038
6c	124713	39058	38272	47383	124712	39058	38272
6d	113400	33314	34920	45166	113161	33269	34919
100µl NY Arg-Sp							
1a	123668	35372	35113	53183	120767	32841	35077
1b	124744	44023	38910	41811	124744	44023	38910
1c	130547	36350	45429	48768	129831	35753	45426
1d	126219	35801	38893	51525	124948	34530	38893
2a	105876	25926	35273	44677	104132	25886	33570
2c	117069	36789	26843	53437	117069	36789	26843
2d	111878	23805	40486	47587	111841	23768	40486
40µl NY Lys-Sp							
3a	115023	32262	31736	51025	115023	32262	31736
3b	121776	29348	37310	55118	121727	29304	37310
3c	111198	29507	36827	44864	111193	29507	36822
3d	121931	32311	40903	48717	121581	32260	40903
4a	143301	38156	47566	57579	143301	38156	47566
4b	119838	34454	38820	46564	119563	34179	38820
4c	115734	29859	42965	42910	115680	29805	42965
4d	131190	30272	44097	56821	130821	29903	44097
100µl NY Lys-Sp							
5a	139039	35822	42890	60327	135318	35785	42593
5b	173387	55564	52818	65005	166787	50562	52818
5c	138038	32945	48634	56459	137250	32157	48634
5d	154441	45920	50230	58291	153752	45920	50230
6a	136961	45557	44422	46982	136961	45557	44422
6b	114818	28253	38473	48092	114604	28238	38473
6c	128546	34233	42435	51878	128542	34229	42435
6d	136875	41264	44126	51485	133924	40268	42171

DG damag	% CA1 dam	%CA3 dam	%DG dama	%Total Damage
64956	46.586963	58.8080154	130.893703	83.1494634
42021	53.3835936	30.0704316	73.2621999	55.0816476
42402	50.9730518	90.4757778	89.798598	78.0603394
37949	40.3043692	26.283275	72.796854	49.5130897
45389	37.5369458	16.6309588	87.9546556	53.1895144
51711	59.3941041	25.6256133	96.746492	63.8752385
42145	39.7280008	30.1924417	97.673179	56.457591
47544	28.7439333	18.9458286	88.648568	48.8585624

Control	CA1	CA3
mean	44.58137	37.129043
sd	9.8855484	25.112753
sem	3.7363861	9.4917286

56615	99.9768966	93.2832209	99.6918472	97.9911173
60439	99.9193262	99.9776431	97.0814058	98.6577181
59821	100	100	99.9882998	99.9944785
62636	95.8178959	100	100	98.8951171
50850	99.9196333	100	100	99.978322
47645	100	99.7266573	99.3597764	99.6659243
47382	100	100	99.9978895	99.9991982
44973	99.8649217	99.9971363	99.5726874	99.7892416

40ul arg	CA1	CA3
mean	99.437334	99.123082
sd	1.4633132	2.3615512
sem	0.5530804	0.8925824

52849	92.8446229	99.8974739	99.3719798	97.6542032
41811	100	100	100	100
48652	98.3576341	99.9933963	99.7621391	99.4515385
51525	96.4498198	100	100	98.9930201
44676	99.8457147	95.1719445	99.9977617	98.3527901
53437	100	100	100	100
47587	99.8445705	100	100	99.9669283

100ul arg	CA1	CA3
mean	98.191766	99.294688
sd	2.697547	1.8183517
sem	1.1012689	0.742339

51025	100	100	100	100
55113	99.850075	100	99.9909286	99.9597622
44864	100	99.986423	100	99.9955035
48418	99.842159	100	99.3862512	99.7129524
57579	100	100	100	100
46564	99.2018343	100	100	99.7705235
42910	99.81915	100	100	99.9533413
56821	98.7810518	100	100	99.7187286

40ul lys	CA1	CA3
mean	99.686784	99.998303
sd	0.4501057	0.0048002
sem	0.170124	0.0018143

56940	99.8967115	99.3075309	94.3855985	97.3237725
63407	90.9977683	100	97.5417276	96.1934862
56459	97.6081348	100	100	99.4291427
57602	100	100	98.8179993	99.5538749
46982	100	100	100	100
47893	99.9469083	100	99.5862098	99.8136181
51878	99.9883154	100	100	99.9968883
51485	97.5862737	95.5695055	100	97.8440183

100ul lys	CA1	CA3
mean	98.253014	99.35963
sd	3.1210166	1.550493
sem	1.1796334	0.5860313

DG	Total
92.221781	61.023181
18.202629	13.016167
6.8799469	4.9196488

DG	Total
99.461488	99.37139
0.9918104	0.7609595
0.3748691	0.2876156

DG	Total
99.875983	99.20264
0.2392107	0.9202803
0.0976574	0.3757029

DG	Total
99.922147	99.888851
0.2165581	0.1304682
0.0818513	0.0493124

DG	Total
98.791442	98.76935
1.9801895	1.451288
0.7484413	0.5485353